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Minireview

Considering Nuclear Compartmentalization in the Light of Nuclear Dynamics

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Many proteins are concentrated in compartments within the nucleus. Chromatin is also compartmentalized at different nuclear sites. However, nuclear proteins have now been shown to be highly mobile. This review considers the formation and function of nuclear compartments in a situation in which proteins are rapidly moving through the nuclear volume.

The nucleus appears to be highly ordered, with molecules involved in common pathways concentrated together in the same sub-nuclear compartments and different chromatin regions adopting distinct sub-nuclear localizations. However, compartmentalization in the cytoplasmic sense, where factors are concentrated behind membranes into isolated microenvironments to enhance biochemical reactions, does not apply in the nucleus. Fluorescence recovery after photobleaching (FRAP) experiments have shown that most nuclear proteins are highly mobile and the interaction of proteins with chromatin and nuclear compartments, once thought to be stable, is now considered highly dynamic. Hence, structural organization within the nucleus represents a dynamic steady state rather than a static situation. Our instinct is to infer function from this structural organization, but this may be flawed in the light of a growing body of evidence suggesting that compartments are themselves established by particular nuclear functions. In this review, we shall analyze this problem from the perspective of chromosome organization. First, by considering how the nuclear environment might influence transcriptional activity and second, by looking at how the nuclear organization of chromosomes relates to that of transcription.

Compartmentalization of Chromatin

In the nucleus, each chromosome occupies its own distinct region or "territory". In the nuclei of many vertebrate species, chromosomes with a low gene density reside at the nuclear periphery, whereas chromosomes with high gene density are located in the nuclear interior (reviewed by Parada and Misteli, 2002). The inactive X chromosome of female mammals also appears to locate at the nuclear periphery. This organization may be paralleled in organisms as distant as yeast, where silenced regions are tethered to the nuclear periphery.

While a spatial organization of chromatin in the nucleus is well established, its functional significance is unclear. Transcriptional silencing might occur if a locus were sequestered at a compact chromatin domain, where transcriptional activators might be sterically excluded or where silencing factors are concentrated. In contrast, transcriptional activation could be facilitated if a locus were relocated to an open, more accessible environment. However, in only a few cases has sub-nuclear position been directly shown to influence gene expression. In *S. cerevisiae*, silencing is restored to a crippled mating-type locus by artificially tethering it to the nuclear envelope (Andrulis et al., 1998). There are correlations between gene silencing and sub-nuclear localization in other organisms. In the *Drosophila brown* dominant (*bwD*) mutation, insertion of a large block of heterochromatin into one allele of the *brown* gene results in the relocation of the wild-type allele to a heterochromatic site and renders it silent. Individual silenced genes in mouse lymphocytes have been demonstrated to occupy distinct sub-nuclear positions—in the vicinity of blocks of heterochromatin formed from satellite repeats (Brown et al., 1997). In these cases, it is not known whether sub-nuclear position is a cause or consequence of silencing. In addition, association with heterochromatin is not observed for all silent loci and is not necessarily incompatible with gene expression (reviewed in Dillon and Festenstein, 2002).

Restricting Mobility and Access

If sub-nuclear position of a locus contributes to the regulation of gene expression, then the ability of chromatin to move around the nucleus must be compatible with this. The most mobile loci studied so far in human nuclei have an average range of movement of 0.5 μm (Chubb et al. 2002). This restricts a locus to only ~1/1000th of the nuclear volume, substantially limiting the range of environments that it has a reasonable likelihood of accessing (Figure 1). However, similar levels of short range rapid chromatin motion seen in *S. cerevisiae* and *Drosophila* give a locus access to a much larger proportion of the nuclear volume (0.5 μm is half the radius of a yeast nucleus) (reviewed by Gasser, 2002). Over longer time periods movements of several microns have been observed in *Drosophila* embryogenesis and spermatogenesis (Vazquez et al., 2001), and human centromeres appear to be particularly mobile during early G1. We conclude that a situation in which chromatin is generally constrained in its motion, but in which large movements can occur in specific situations, is not incompatible with a model whereby nuclear position regulates gene expression.

Models where localization near silent domains brings about gene inactivation usually presuppose that heterochromatin restricts access to transcriptional activators and/or that it provides a local concentration of silencing factors. It is assumed that the structure of heterochromatin is in some way more compact than that of other regions. Biophysically, heterochromatin certainly does seem to have a distinct higher order structure and shows reduced accessibility to exogenous nucleases (Dillon and Festenstein, 2002). However, there is little evidence that heterochromatin provides an access barrier to either chromatin proteins or transcription factors. The average residence time for histone H1 is the same (3.5

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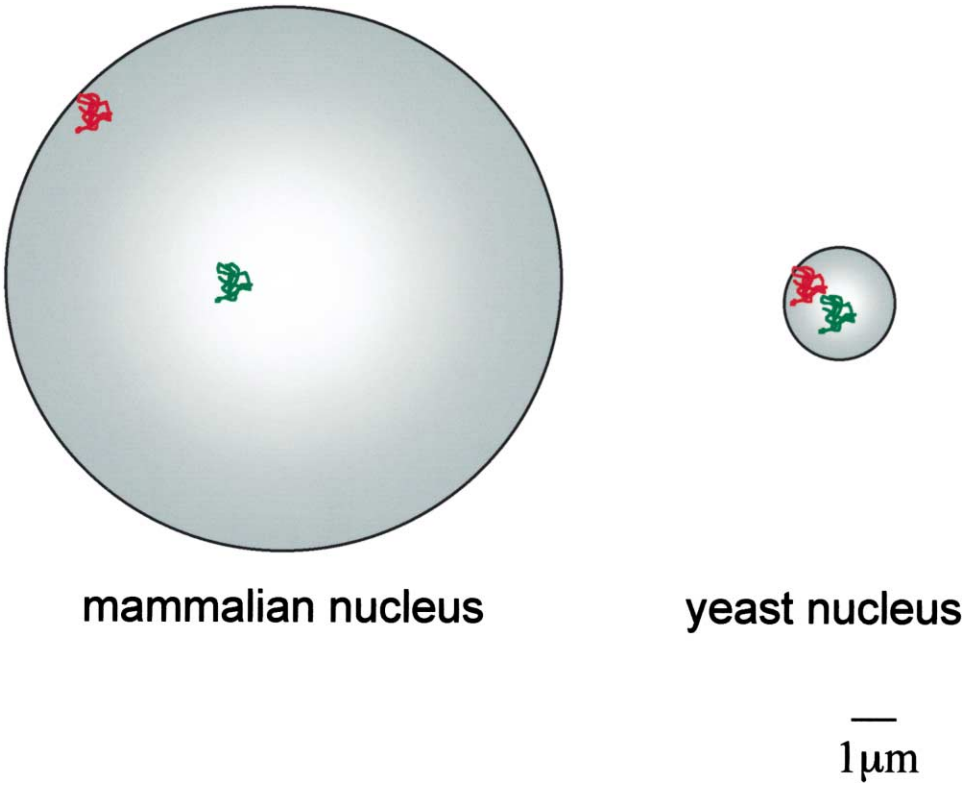

Figure 1. Constrained Chromatin Motion in an Organized Nucleus

The random, but constrained (0.5 μm average range) chromatin motion of a locus in both mammalian and budding yeast nuclei allows it to sample only a small proportion of the nuclear volume. A locus positioned close to the nuclear periphery (red) will sample a local environment where silencing proteins (gray) might be at increased concentration. A locus distant from the nuclear periphery (green) is less likely to encounter regions of high silencing protein concentration during its wandering.

min) at both heterochromatic and euchromatic sites in the nucleus, although there may also be a larger fraction of immobile H1 at heterochromatin (reviewed by Misteli, 2001). Some transcription factors can also access transgenes located in heterochromatin (Dillon and Festenstein, 2002).

Most nuclear proteins are highly mobile, able to cross the nucleus in a few tens of seconds; even fluorescent dextrans up to 580 kDa in mass (the effective size of which is larger than most polypeptides) are freely mobile in mammalian nuclei. However, fluorescent dextrans of 2000 kDa are relatively immobile, so is chromatin inaccessible to large protein complexes? The nuclear movements of mRNP particles suggest they are confined to regions of the nucleus, which contain reduced amounts of chromatin as indicated by DNA staining. This biophysical evidence for limited protein accessibility to chromatin is a cornerstone of one popular model for a functional nuclear architecture, the chromosome territory—interchromatin compartment (CT-IC) model (reviewed by Cremer and Cremer, 2001). The model proposes that transcription complexes are established in, and restricted to, the interchromatin compartment. Active genes are positioned on the surface of chromatin domains that line the interchromatin compartment and so are accessible for transcription. Silenced genes are located within the interior of compact chromatin domains and require chromatin remodeling events for repositioning and activation. However, active genes are not necessarily at the periphery of chromosome territories or the surface of sub-chromosomal domains (Mahy et al., 2002a). Moreover, the presence of very large protein complexes, e.g., RNA polymerase holoenzymes, roaming an interchromatin compartment is questioned by a FRAP study of the assembly dynamics of the RNA polymerase I transcription machinery (Dundr et al., 2002). It is suggested that assembly of a productive PolI elongation complex occurs by stochastic binding of individual polymerase subunits at the site of transcription, not by binding of a pre-assembled holoenzyme. The dynamics of RNA polymerase II and transcriptional regulators are consistent with a similar mechanism occurring for transcription of most genes in the nucleus (Kimura et al., 2002; Becker et al., 2002), but this needs to be reconciled with the considerable biochemical evidence for the existence of holoenzyme complexes.

The Importance of Local Concentration

Because of the essentially stochastic nature of the binding of individual proteins, these FRAP studies indicate that the assembly of a functional transcription elongation complex, which is dependent on a large number of individual rate constants, is an inefficient, possibly rate limiting, process. The overall flux of the reaction may therefore be extremely sensitive to any factors that affect protein binding and dissociation, such as histone modifications (effects on protein binding), nucleosome

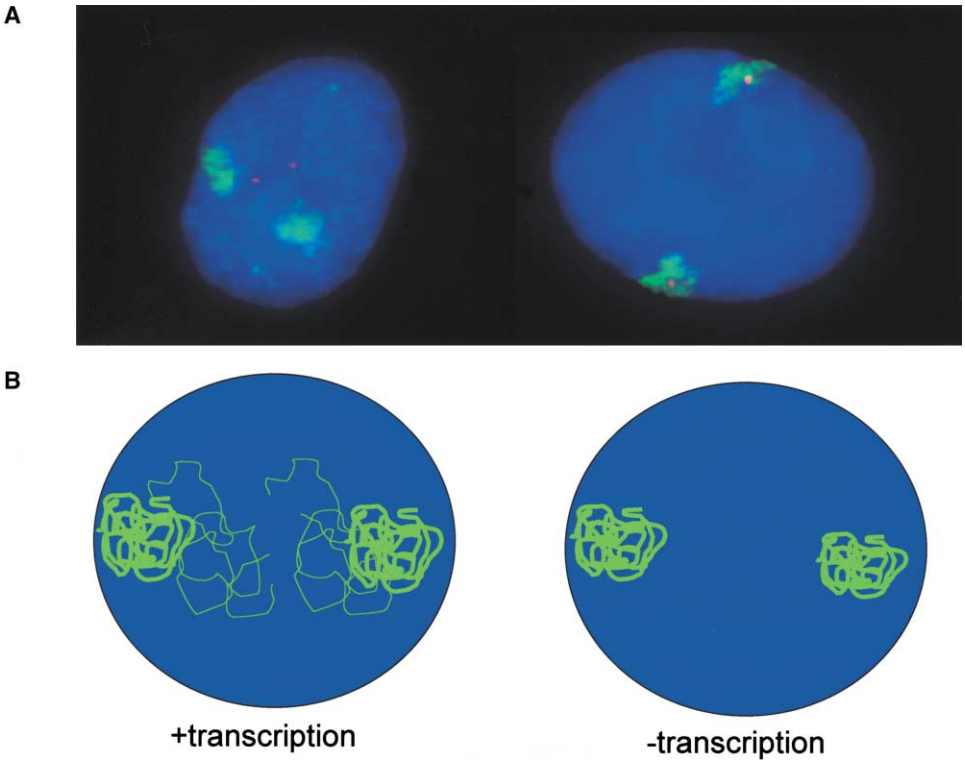

Figure 2. Transcription Decondenses Chromatin

(A) Loci (red) in areas of very high transcriptional activity are frequently found outside of chromosome territories (green). When transcription is stopped (for example with an inhibitor), loci are now found more frequently within chromosome territories (Mahy et al., 2002b).

(B) It is suggested that transcription decondenses chromosome territories, extruding large loops of chromatin that then collapse back into condensed territories when transcription ceases.

positioning (effects on local accessibility and binding), and also the local concentration of both protein and target. This is where nuclear compartmentalization might have large affects on the efficiency of nuclear processes. Even with factors in constant flux, localization of a gene at a particular nuclear site might expose it to elevated or decreased concentrations of a protein, potentially altering the probability of binding or complex formation. The clustering of rRNA genes into a nucleolus might increase the efficiency of their transcription by providing a high local concentration of productive binding sites (promoters) for the RNA polymerase subunits. In turn, the elevated concentrations of RNA polymerase subunits and transcription factors in the nucleolus increases the likelihood that they will encounter another rRNA promoter rather than a non-specific DNA binding site. The same arguments could apply to gene silencing. Localization of a gene at the nuclear periphery or in the vicinity of heterochromatin might expose it to elevated concentrations of a silencing protein (Figure 1) or to decreased concentration of a transcriptional activator.

The importance of concentrating factors in a particular nuclear compartment can be assessed in situations where certain nuclear compartments are absent or disrupted. The nucleolus is not required for ribosomal gene transcription. Transcription of rRNA initiates in telophase prior to the formation of mature nucleoli, and rRNA transcription persists in the presence of chemical inhibitors of nucleolar formation. However, preribosomal processing is defective in this situation (Sirri et al., 2002). PML bodies are nuclear structures enriched in transcriptional regulators and enzymes for protein and nucleic acid catabolism. PML bodies are lost in mice lacking PML protein and the other proteins normally concentrating in this nuclear compartment are dispersed throughout the nucleoplasm. The mutant animals are viable and fully fertile but display effects on cell growth, tumorigenesis, and the differentiation of hematopoietic precursors (Wang et al., 1998). Cajal bodies (CBs) are enriched in factors required for the biogenesis of small nuclear ribonucleoproteins (snRNPs). Mutant mice lacking p80 coilin have residual CBs that fail to recruit much of the snRNP processing machinery. The mutant animals are viable, although their numbers are reduced in inbred backgrounds (Tucker et al., 2001). The analysis of PML and p80 coilin mutants does not determine whether their phenotypes are caused by the loss of compartmentalization or the absence of PML or p80 coilin protein function, but they do demonstrate that intact PML and Cajal bodies are not essential for cell viability. It is clear from these studies that many nuclear reactions can proceed in absence of compartmentalization, although compartments may enhance the efficiency of these processes.

Does Function (Transcription) Drive Organization?

So far we have discussed how nuclear organization might impact on function (e.g., gene regulation). Can function also drive organization? Transcription (or the lack of) does appear to be able to influence nuclear

compartmentalization. The inhibition of either RNA polymerase I or II transcription causes a dispersal of nucleoli. Conversely, the induction of rDNA transcription on episomes induces the formation of mini-nucleoli on the foreign DNA that generate mature and functional ribosomes.

Although RNA polymerase II transcribed genes can be expressed from within chromosome territories, it is now clear that many chromosomal regions localize outside the visible confines of chromosome territories. Although the number of loci that have been analyzed is small, all the reported incidences of this occur at gene dense and transcriptionally active regions (Mahy et al., 2002b). The major histocompatibility (MHC) locus at 6p24 is observed on loops of chromatin that extend away from the human chromosome 6 (Volpi et al., 2000). The epidermal differentiation complex (EDC) at 1q21 is similarly located outside the chromosome 1 territory. "Looping out" of these loci is more pronounced in cells in which the coordinately regulated genes from these regions are expressed. The gene dense 11p15.5 region also frequently extends out from the chromosome 11 territory and the use of transcriptional inhibitors showed that this was partly dependent upon ongoing transcription (Mahy et al., 2002b) (Figure 2). These observations suggest that the architecture of chromosome territories is the product of transcription rather than a structure set up to aid it. The mechanism through which transcription could extend out large (many Mb long) extents of chromatin from chromosome territories is unknown but could include, for example, passage of the elongating RNA polymerase itself, or associated activities such as chromatin remodeling, or nucleosome modification. It is interesting that the retraction of looped out 11p15.5 chromatin is only partial when transcription is blocked. Does this suggest that there are other forces maintaining the extended state? An alternative possibility is that constraints on chromatin motion prevent total retraction of the looped out regions.

If transcription drives a decondensation of chromosome territories, then in its absence, chromatin would adopt a condensed default state (Figure 2). Indeed, a condensation of the human chromosome 19 territory is seen by light microscopy when transcription is inhibited, and by electron microscopy chromatin fibers at Balbani ring genes thicken and form condensed chromatin when transcription is blocked (Andersson et al., 1984). The nuclei of many terminally differentiated cells show large domains of compact chromatin and this has been interpreted as the formation of a specialized chromatin structure (facultative heterochromatin) to aid and maintain the global shut down of gene expression. However, it might merely be what happens to chromosomes when the bulk of gene expression stops. A recent study by Hediger et al. (2002) suggests that silent chromatin may also have the ability to drive nuclear organization. Budding yeast telomeres are anchored to the nuclear periphery by redundant mechanisms. In the absence of telomeric silencing factors, the Ku protein is responsible for telomeric anchoring. In the absence of Ku, silent chromatin itself can mediate attachment.

We have discussed the evidence that interphase chromosome architecture reflects a functional organization of chromatin, where the nuclear environment of a locus influences its transcriptional activity. We have also outlined an alternative view, where the establishment of chromosome architecture is a consequence of transcriptional activity. These two views are not mutually exclusive; transcription may drive the establishment of nuclear order, but the order itself may facilitate the control of transcription.

An influence of chromatin structure on transcription by steric exclusion seems inconsistent with the demonstrated accessibility of chromatin to mobile nuclear proteins. Transcription factors have the freedom of the nucleus and are small enough to access any environment. We have argued that differences in local protein concentrations at different nuclear sites may be sufficient to influence gene expression or gene silencing. However, these issues can only be resolved by perturbing nuclear environments and analyzing the downstream effects. The development of technologies to look at chromatin, genes, and their expression products in living cells will certainly facilitate this.

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